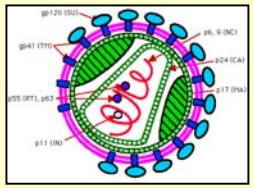
Identification of an Inhibitory Binding Site to HIV-1 Integrase with Affinity Acetylation and MS

Nick Shkriabai[1], D. Eric Anderson[2], Sachindra S. Patil[3], Sonja Hess[2], Terrence R. Burke Jr. [3], Mamuka Kvaratskhelia*[1]

[1] The Ohio State University Health Sciences Center, College of Pharmacy, Center for Retrovirus Research and Comprehensive Cancer Center, Columbus, OH 43210, [2] Laboratory of Medicinal Chemistry, National Cancer Institute, NIH, [3] Proteomics and Mass Spectrometry Facility, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, DHHS, Bethesda, MD 20892.

Objective

To identify the inhibitory binding site to HIV-1 Integrase



Introduction

HIV-1 Integrase (HIV-IN) is one of the three enzymes essential for virus replication. With the increasing resistance towards current HIV treatments, HIV IN has become an important target for the development of new drugs. For a rational drug development, knowlegde of the structure of the targeted enzyme is imperative. However, tremendous efforts to obtain a structure of full-length protein have been impeded by poor protein solubility. To overcome this bottleneck, we have developed a methodology that combines affinity acetylation and MS analysis. HIV IN has been reacted with an a caffeoyl derivative containing aryl di-O-acetyl groups capable of effectively acetylating Cys, Lys and Tyr residues. This approach allowed us to characterize inhibitor : HIV IN interactions.

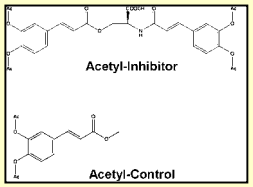


Figure 2. Structures of Acetyl-Inhibitor and Acetyl-Control.

Experimental Design

Full-length integrase (F185K/C280S) was expressed in *E. coli* and purified. Acetylation of HIV-1 IN was carried out in 50 mM HEPES, pH 8.0 buffer. The protein was treated with DTT and subjected to SDS-PAGE. The integrase band was excised and digested with 1 µg trypsin. Proteolytic peptide fragments were subjected to MS and MS/MS analyses using a Waters Q-ToF-II instrument equipped with an electrospray source and a Waters cap-LC. The peptides were separated with a precolumn and a C18 column using two sequential linear gradients of 5-40% and 40-90% of acetonitrile for 35 and 25 min, respectively. A Model of the IN:Acetylated-Inhibtor complex was generated and minimized using the Builder module of InsightII (ver 2000.1, Accelrys).

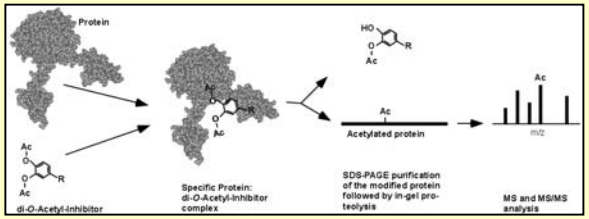


Figure 2. An aryl di-*O*-acetyl-containing inhibitor is incubated with target protein. Formation of a specific complex results in covalent modification of the protein at the inhibitor binding site. Acetylated peptide fragments and individual residues are identified from MS and MS/MS analysis.

Results

Our experimental strategy was to accurately map the inhibitor binding site of HIV IN using affinity acetylation and mass spectrometry. For this purpose, we employed a known HIV-1 integrase inhibitor containing aryl di-*O*-acetyl groups (Acetylated-Inhibitor). In addition, we designed a control compound (Acetylated-Control) that also contained an aryl di-*O*-acetyl group but did not inhibit HIV-1 integrase. Mass spectrometric examination of the reactivity of these compounds with a model peptide library, which collectively contained all 20 natural amino acids, revealed that aryl di-*O*-acetyl compounds effectively acetylate Cys, Lys and Tyr residues.

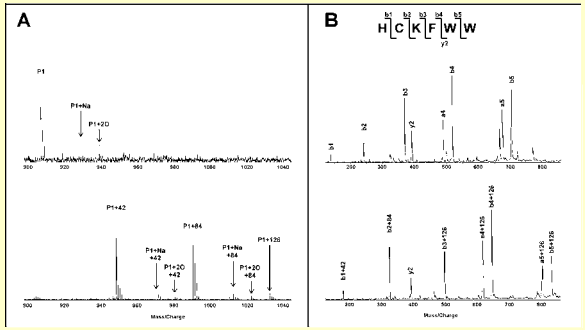


Figure 3. MALDI-ToF data on acetylation of peptide HCKFWW. (**A**) MS data of the peptide prior to and after treatment with Acetyl-Inhibitor. New adducts formed: +42; +84 and +126 corresponding to the addition of 1, 2 and 3 acetyl groups, respectively. Small amounts of sodium adduct (+Na) and an oxidized species (+2O). (**B**) MS/MS data of the unmodified peptide HCKFWW (top panel) and the acetylated peak +126 (bottom panel).

Acetylated-Inhibitor and Acetylated-Control exhibited comparable chemical reactivity with respect to these small peptides. However, these two compounds differed markedly in their interactions with HIV-1 integrase. In particular, Acetylated-Inhibitor specifically acetylated K173 at its inhibitory concentration (3 µM), while this site remained unrecognized by Acetylated-Control.

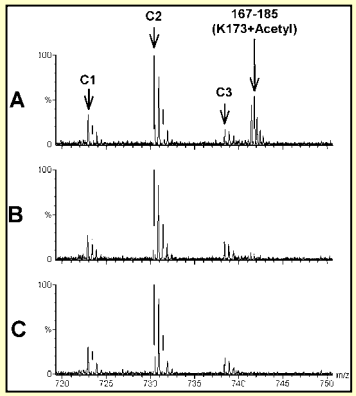


Figure 4. Mass spectrometric data showing specific acetylation of HIV-1 IN with Acetyl-Inhibitor. (**A**) IN+3 µm Acetyl-Inhibitor, (**B**) IN+3 µm Acetyl-Control, (**C**) free IN. MS spectra illustrate that treatment of IN with 3 µM Acetyl-Inhibitor resulted in a single new peak: 167-185+Acetyl (A, top panel). The molecular weight of this triply charged ion peak (741.03^{3+} = 2220.13 Da) corresponds to the 167-185 peptide (MW=2178.12 Da) plus one acetyl group (MW=42.03 Da).

Our data enabled creation of a detailed model for the integrase:Acetylated-Inhibitor complex, which indicated that the inhibitor selectively binds at an architecturally critical region of the protein. The methodology reported herein has a generic application for systems involving a variety of ligand protein interactions.

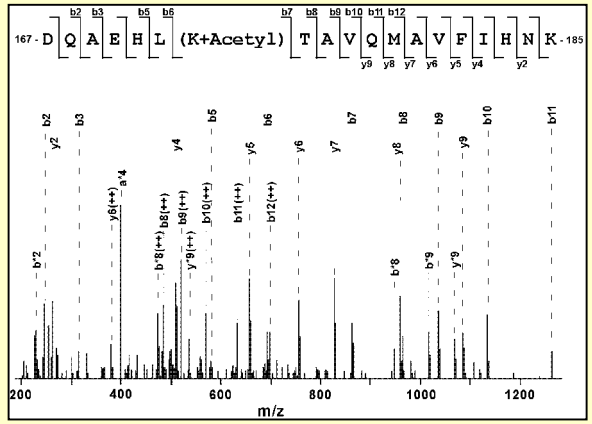


Figure 6. MS-MS data on the acetylated IN peptide (167-185+Acetyl). The mass increment between "b6" and "b7" ions corresponds to an acetylated lysine (K173+Acetyl).

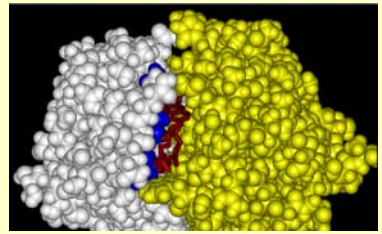


Figure 7. A model of IN:Acetyl-Inhibitor complex.

Conclusions

Taken together, we have been able to accurately map the inhibitor-protein interactions of HIV-IN and the Acetyl-Inhibitor. This technology has a generic application for studying a wide variety of inhibitor-protein interactions. The ability to perform high-resolution structural analysis of inhibitor-protein complexes under biologically relevant conditions using only very limited sample quantities makes such a mass spectrometric approach very attractive and opens a doorway for implementing high-throughput schemes.